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The limit of mass determination with an AFM cantilever-based system

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Nowadays, modern nanotechnology-based analytical systems employing molecular detectors - such as atomic force microscope (AFM) - are being developed. Among nanotechnology-based systems, one can distinguish nanomechanical cantilever-based ones, which allow one to operate with condensed matter at the nanoscale, thus enabling determination of various characteristics (mass [1], elasticity [2] etc.) at the level of single macromolecules or nanoparticles. In these systems, the target objects are adsorbed from the volume of analyzed sample onto the cantilever, and the resonance frequency of the AFM cantilever (which decreases in a linear proportion to the added mass) is measured [3]. The adsorption of target objects increases the mass of the cantilever; this causes shift in the resonance frequency of the cantilever, which is measured by AFM electronics. As target objects, nanoparticle-labeled single molecules of protein markers of diseases can be used [3].

In our present study, the sensitivity of a microcantilever system employing standard AFM microcantilevers for measurements in vacuum, has been estimated. We have demonstrated that such a system allows one to register ~100 gold nanoparticles (AuNPs), what corresponds to 17 μL of 10^{-17} M solution of protein labeled with these nanoparticles. The convenience of this approach consists in that no additional equipment (such as atomic weights [1,4]), is required to measure the mass of the adsorbed protein.

To measure the resonance frequency shift caused by adsorption of nanoparticles onto the cantilever, the following technique was employed. AFM cantilever (PPP-CONTR-50, Nanosensors Inc., USA; resonance frequency 6 to 21 kHz; height 450±10 μm; width 50±7.5 μm; thickness 2±1 μm; force constant 0.02 to 0.77 N/m), whose resonance frequency was measured prior to the experiment, was incubated in a drop of solution containing 20-nm-diameter AuNPs during 10 min. After the incubation, the cantilever was dried and placed in the vacuum chamber of an NTEGRA Aura AFM (NT-MDT, Zelenograd, Russia), and its resonance frequency was measured and compared with that before the incubation. All measurements have been performed in vacuum at 0.1 Torr. The mass of the AuNPs adsorbed onto the cantilever was estimated from the resonance frequency shift according to Kosaka et al [3]:

$$\Delta m = \frac{2m(f_1 - f)}{f},$$

where f and f_1 are the resonance frequencies of the cantilever before and after the incubation, respectively; m is the mass of the cantilever; and Δm is the mass of the adsorbed AuNPs.

Therefore, given the mass of a single AuNP, the total number of the AuNPs adsorbed onto the cantilever can be determined. The size of AuNPs and the number of AuNPs adsorbed onto the cantilever were estimated by scanning electron microscopy (SEM) visualization of the cantilever with adsorbed AuNPs employing a Hitachi S5500 electron microscope (Hitachi, Japan).

Since the resonance frequency shift for a 20-kHz cantilever, registerable with the system employed in our study, is 20 Hz (what amounts to 0.1% from 20 kHz), the mass of the adsorbed AuNP causing this shift must be 1000 times smaller than that of the cantilever itself. Let us estimate the mass of the silicon (of 2.33 g/cm^3 density) cantilever:

$$m=\rho V=2.33\ \text{g/cm}^3(450\times50\times1\times10^{-12})\ \text{cm}^3=5.24\times10^{-8}\ \text{g}.$$

For such a cantilever, 0.1% mass increase ($\Delta m/m$=0.001) corresponds to Δm of the order of 10^{-10} g. Since the masses of 20-nm and 100-nm AuNPs make up $6\text{x}10^{-16}$ g and $6\text{x}10^{-14}$ g, respectively, the number of these AuNPs causing $\Delta m=10^{-10}$ g corresponds to ~10^6 (for 20-nm

AuNPs) and ~10^4 (for 100-nm AuNPs). Fig. 1 displays the data obtained in our experiments. These data have indicated that adsorption of 10^6 of 20-nm AuNPs caused 20-Hz shift in the cantilever's resonance frequency (what is in a good agreement with the theoretical estimations) - in contrast to the case with 10^4 of 20-nm AuNPs, when no shift in the resonance frequency was registered.

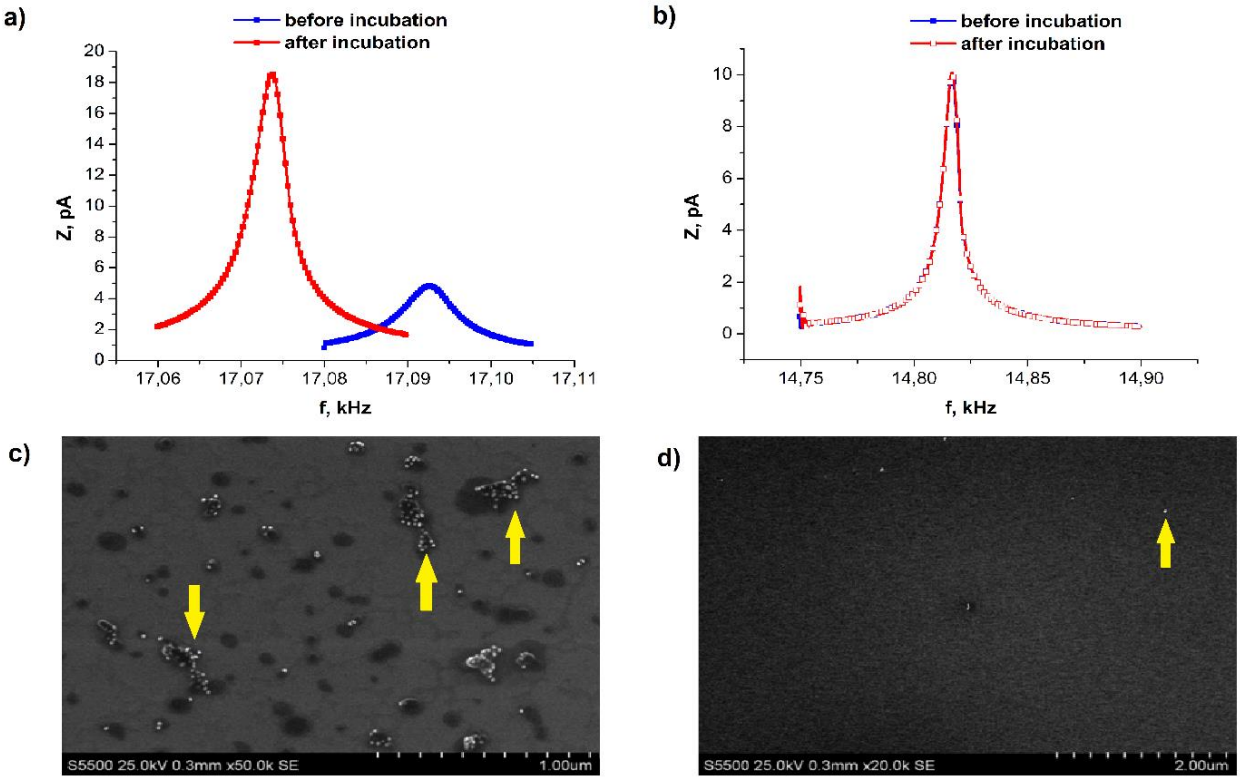

Figure 1. The resonance frequency curves of the AFM cantilever obtained before (red curves) and after (blue curves) their incubation in AuNP-containing solution, and corresponding SEM images of single AuNPs adsorbed onto the cantilever surface, in the case of adsorption of 10^6 (a, c) and 10^4 (b, d) 20-nm AuNPs.

Due to high sensitivity of mass determination, the use of cantilever-based method for protein detection in biomedical applications is promising. To date, modern techniques allow labeling of protein molecules with AuNPs in 1:1 ratio [5]. This fact gives an oportunity for the detection of so-labeled protein molecules with high sensitivity. In this way, using 20-nm AuNPs for this purpose, the 10^{-15} M protein detection limit can be attained upon analysis of 1 mL of sample. Moreover, if 100-nm AuNPs will be used instead of 20-nm ones, the detection limit of labeled protein molecules can be shifted down to 10^{-17} M.

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